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## Albuterol aids resistance exercise in reducing unloading-induced ankle extensor strength losses

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**Caruso, John, John Hamill, Miki Yamauchi, Dean Mercado, Tim Cook, Brian Higginson, Sean O'Meara, Jeff Elias, and Steve Siconolfi.** Albuterol aids resistance exercise in reducing unloading-induced ankle extensor strength losses. *J Appl Physiol* 98: 1705–1711, 2005. First published January 7, 2005; doi:10.1152/jappphysiol.01015.2004.—While resistance exercise (REX) reduces ankle extensor (AE) mass and strength deficits during short-term unloading; additional treatments, concurrently administered with REX, are required to attenuate the greater losses seen with longer unloading periods. Subjects performed left leg REX, which otherwise refrained from ambulatory and weight-bearing activity for 40 days, while randomized to a capsule (placebo, albuterol) dosing regimen with no crossover to note whether albuterol helps REX mitigate unloading-induced AE losses. A third group of subjects served as unloaded controls. On *days 0, 20, and 40*, the following data were collected from the left leg: calf cross-sectional area and AE strength measures. Cross-sectional area was estimated using anthropometric methodology, whereas AE strength data were obtained from eight unilateral calf-press repetitions on an inertial-based REX device. Repeated-measures mixed-factorial 3 × 3 analyses of covariance, with *day 0* values as a covariate, revealed group × time interactions for the strength variables eccentric total work (ETW) and average power (EAP). Tukey's honestly significant difference shows REX-placebo subjects incurred significant ETW and EAP losses by *day 40*, whereas the REX-albuterol treatment evoked strength gains to those same variables without concurrent muscle accretion. Corresponding concentric variables did not display similar changes. *Day 40* control data significantly declined for many variables; relative to the REX-albuterol treatment, some losses were significant after 20 days. ETW and EAP gains to unloaded AE may be due to one or more mechanisms. Continued research identifying mechanisms responsible for such changes, as well as the safety of REX-albuterol administration in other models, is warranted.

salbutamol; triceps surae; unilateral limb suspension; simulated space-flight

UNLOADING, WHEREBY ALL OR PART of the body refrains from normal weight-bearing or ambulatory activity, causes muscle mass and strength losses to disuse (spaceflight, bed rest, etc.) models. Among the muscles most adversely affected by unloading are the ankle extensors (AE), which typically exert force during load-bearing and ambulatory activities (23). Illustrating the deleterious effects of unloading, strength losses after space travel that exceed 10% of preflight values have been deemed operationally significant and may compromise a crew-member's ability to perform emergency egress (1). Human AE

mass and strength losses (Fig. 1) vary by the duration of unloading, with longer periods evoking deficits far in excess of 10% of preflight values. Thus reducing AE deficits is needed to preserve in-flight mass and strength and to prepare for post-flight resumption of weight-bearing and ambulatory activity.

Unloading-induced losses result from a lack of mechanical loading. Thus activities that mechanically load the AE during unloading may best preserve mass and strength (23). Aerobic exercise administered during space travel minimally reduced muscle mass and strength losses (51). However, resistance exercise (REX), a form of physical activity that offers a more intense and specific mechanical loading stimulus, preserved AE mass and strength in humans during short-term (14–21 days) unloading versus unloaded controls (CTRL) (3, 6, 46). However, REX as a sole countermeasure during longer unloading periods (32, 33, 51), which note far greater losses, may not prove as effective (23, 44). Thus a broader approach beyond mechanical loading to also include pharmacological agents may best reduce unloading-induced mass and strength losses (23, 44). One such candidate is the  $\beta_2$ -agonist albuterol.

$\beta_2$ -Agonists increase muscle mass and strength in weight-bearing and unloaded animal models (28). However, the  $\beta_2$ -agonist clenbuterol also caused harmful side effects in animals when given in quantities far greater than comparable human dosages, with such changes preceded by adverse blood pressure and heart rate perturbations (17, 28, 47). Yet physiological ( $10 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) clenbuterol dosing in aging rats did not improve muscle mass and strength (15). A 3-wk oral albuterol (salbutamol) assignment (16 mg/day), the maximal Food and Drug Administration-approved oral therapeutic dosage, caused modest strength gains in sedentary humans (39). Yet the same dose in ambulatory humans evoked greater strength gains with a concurrent REX program without adverse side effects versus subjects given a REX-placebo treatment (13, 14). Although promising, perhaps even greater relative gains would occur in unloaded models, which note heightened  $\beta$ -receptor density and responsiveness (19). However, the magnitude of long-term unloading-induced AE mass and strength losses (32, 33, 51) exceed gains provided by  $\beta_2$ -agonists alone (38, 39). Also, since it does not provide a mechanical loading stimulus, albuterol as a sole countermeasure offers little hope for reducing AE mass and strength losses.

Prior studies quantified unloading-induced AE mass and strength losses and the merits of concurrent REX to reduce

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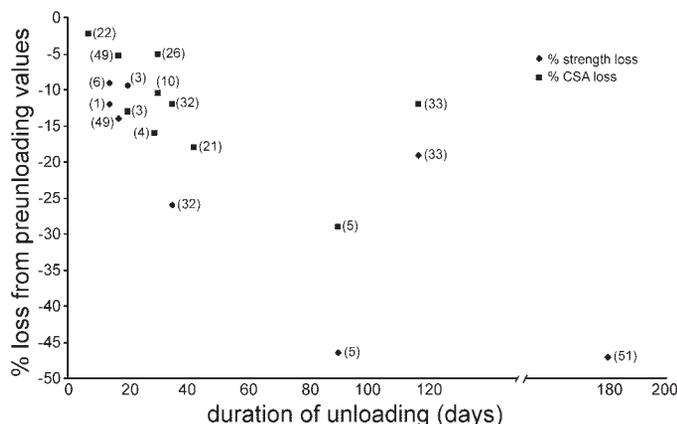


Fig. 1. Ankle extensor mass and strength losses with unloading. Numbers in parentheses are reference numbers.

such changes during short-term unloading. The present study examines whether albuterol provides an additive effect, beyond that seen with REX alone, on AE mass and strength loss attenuation during 40 days of unloading in healthy humans. The present study also examines whether albuterol causes harmful heart rate and blood pressure changes. We hypothesize that, during 40 days of unloading, a REX-albuterol treatment will reduce unloaded lower leg mass and strength losses to a greater extent than REX-placebo or CTRL assignments, without causing adverse resting blood pressure or heart rate changes.

## MATERIALS AND METHODS

Healthy untrained subjects ( $n = 48$ ; 26 men, 22 women) provided medical records and informed, written consent. Local human subject committees approved all procedures. Subjects were asymptomatic for asthma, cardiovascular disease, hypertension, hyperthyroidism, glucose-3-phosphate enzymatic deficiencies, hypersensitivity to sympathomimetic amines, tachycardia, diabetes mellitus, and convulsive disorders. In addition, subjects did not take monoamine oxidase inhibitors or ephedrine-based compounds and were free of musculoskeletal injuries. With a double-blind protocol, subjects were randomized to an albuterol or placebo capsule treatment with no crossover. A third group served as unloaded CTRL subjects. During their participation, female subjects took oral contraceptives to reduce natural strength fluctuations to minimize their impact on study results (45).

**Unilateral limb suspension.** To stimulate unloading-induced mass and strength losses, all subjects performed 40 continuous days of unilateral limb suspension (ULLS), whereby their left leg refrained from normal weight-bearing and ambulatory activity. ULLS was performed with a 5-cm rubber sole attached to the bottom of each subject's right shoe and a pair of crutches to ensure the left lower leg refrained from exerting or incurring weight-bearing forces (1, 46). Compliance to ULLS was examined daily and deemed to be maintained if the unloaded calf was 1–2°C cooler and 1–2 cm larger in circumference versus the weight-bearing calf (1, 46).

**REX training.** Performed by subjects randomized to albuterol or placebo capsule dosing, REX workouts were done three times per week during the 40-day period, which consisted of maximal voluntary effort four-set, eight-repetition unilateral calf presses with their left AE and 90-s rests between sets. The only exceptions were the weeks of mid- and posttesting (*days 20* and *40* of unloading) in which subjects trained twice per week. Workouts were done on an inertial-based exercise device (YoYo Technologies, Stockholm, Sweden) that provided concentric and eccentric resistance from a pair of flywheels

and suggested as an in-flight countermeasure to muscle mass and strength losses (9). Subjects plantar flexed and then resisted dorsiflexion against inertial resistance for repetitive concentric and eccentric AE actions. Within 1 wk before unloading, subjects in all three groups performed a familiarization session on the device to become accustomed to its operation since it does not function like standard REX equipment. Data reproducibility from an inertial-based REX device was recently assessed in workouts ( $n = 23$ ) spaced 3 days apart involving concentric and eccentric AE actions (4). Flywheel dimensions equaled those used in the present study. Coefficient of variation (6.8–8.4%) and  $r$  values (0.82–0.88) suggest inertial-based REX measures were accurate and reproducible (4).

Before workouts, REX-albuterol and REX-placebo subjects were checked for ULLS compliance and then had their resting heart rates and blood pressures measured at the right radial and brachial arteries, respectively. Subjects were then weighed and performed a bilateral 5-min stationary bicycle warm-up at 70 rpm against 9.81 N of resistance. Repetitions were done on the inertial-based device with the left knee fully extended to engage the AE across a subject's full range of motion. Two flywheels, joined by an axle, attached to a footplate via a cord to provide inertial resistance. Applying AE shortening forces, subjects began seated calf press repetitions. Kinetic energy, transferred to the flywheel during muscle shortening, reversed the footplates' movement. The AE then exerted muscle-lengthening forces to slow the footplates' returning motion. Thus the AE provided both shortening and lengthening forces per repetition. All REX-albuterol and REX-placebo subjects completing the project were 100% compliant to training.

**Capsule dosing.** A randomized double-blind assignment with no crossover was used to assess whether albuterol had an additive effect, beyond that shown with REX alone, on AE mass and strength loss attenuation. Subjects in two of the three groups received either an albuterol or placebo (lactose) dosing treatment and took up to four capsules daily, one each with a meal or snack during the day. Due to the double-blind assignment, REX-albuterol and REX-placebo subjects both received the following break-in protocol to reduce possible side effects: 1) *unloading days 1* and *2*: 1 capsule/day; 2) *unloading days 3* and *4*: 2 capsules/day; 3) *unloading days 5* and *6*: 3 capsules/day.

From *days 7* to *40*, REX-albuterol and REX-placebo subjects took four capsules daily. For REX-albuterol subjects, this equaled 16 mg/day, the maximal Food and Drug Administration-approved oral therapeutic dosage. REX-albuterol and REX-placebo subjects were reminded at each workout to consume four capsules daily, with those who completed the project reporting 100% compliance.

**CTRL subjects.** CTRL subjects performed the 40-day ULLS protocol but did not partake in treatments (REX, capsule dosing) associated with the other group assignments. CTRL subjects were treated as those using the same model in prior studies (1, 46). CTRL subjects performed all test session procedures, as did volunteers in the other groups.

**Test sessions.** Unloaded lower leg testing occurred on *days 0*, *20*, and *40*, which began by checking subjects for ULLS compliance (1, 46). Like REX training, test sessions then measured body weight, resting heart rate, and blood pressure. Unloaded calf cross-sectional area (CSA) was then examined with established anthropometric methods that provide a valid assessment of muscle mass changes over time (41). Per test session and subject, unloaded calf circumference was measured halfway between the fibular head and lateral malleolus with a cloth tape measure. Four calf skinfold measurements were next taken 90° apart at the circumference site. The four measurements were then averaged and then subtracted from the radius of the circumference site to correct for subcutaneous fat. The corrected radius value was then used to estimate CSA. The principal investigator, using the same equipment per test session, performed all CSA measurements and calculations. Per test session and subject, test-retest unloaded calf CSA estimates deviated <1%.

After a bilateral 5-min stationary bicycle warm-up, testing concluded by measuring unloaded AE strength from eight maximal voluntary effort unilateral calf press repetitions on the inertial-based device. With strength testing done against inertial resistance, flywheel velocity collected online (MP 100, Biopac, Santa Barbara, CA) at 10 Hz determined concentric and eccentric total work (ETW) and average power (CAP and EAP, respectively) with the following equation (9):

$$\text{Kinetic energy} = (1/2)(J)(\omega^2)$$

where  $J$  is rotational inertia and  $\omega$  is angular velocity.

Figure 2 shows a waveform produced from a strength testing repetition with flywheel velocity plotted as a function of time. Starting each repetition, inertia was overcome by AE shortening forces to cause the waveform and flywheel velocity to increase. The high point of the waveform [peak angular velocity (PAV)], achieved with full plantar flexion, was proportionate to the shortening forces exerted. PAV values were inserted into the above equation to measure concentric work, which was summed for the eight repetitions to determine concentric total work; the highest PAV value per set was also collected as an instantaneous measure of concentric AE strength. Kinetic energy transfer then reversed the direction of the footplate as the AE exerted lengthening forces, causing the flywheel velocity and waveform to decline. Differences in peak and lowest angular velocities represent the lengthening forces exerted and determined eccentric work, which was summed for the eight repetitions to calculate ETW with the above equation. The magnitude of flywheel deceleration is limited by the PAV from muscle shortening; thus ETW and EAP values are submaximal and in part dependent on the concentric forces exerted per repetition. CAP and EAP were calculated using a work-to-time ratio and averaged for the repetitions.

**Statistics.** Mean differences for body weight, cardiovascular, estimated calf CSA, and AE strength-dependent variables were compared with  $3 \times 3$  repeated-measures mixed-factorial analyses of covariance with *day 0* values as a covariate and planned orthogonal contrasts with a 0.05 alpha. Planned orthogonal contrasts were used based on prior work that showed  $\beta_2$ -agonists consistently led to greater muscle mass and strength gains than placebo dosing (2, 13, 14, 28, 29). Significant dependent variables were examined with Tukey's honestly significant difference test to determine the source of interaction.

## RESULTS

Attrition resulted from ULLS, whereby subjects performed daily activities on crutches. Misjudging the demands of ULLS,

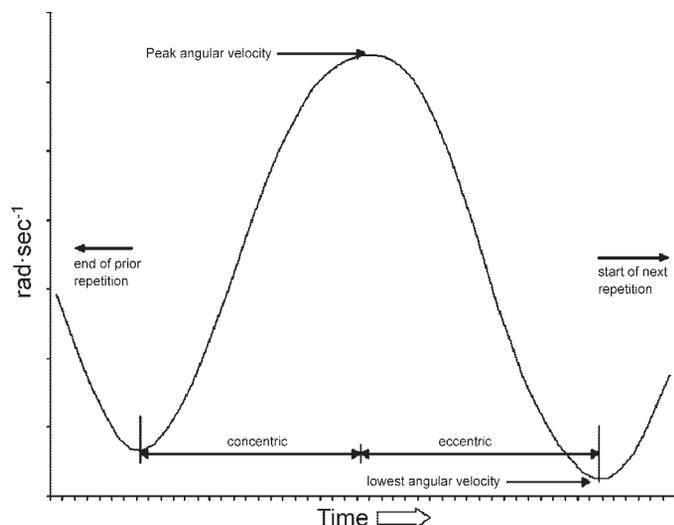


Fig. 2. Waveform from a seated calf press repetition done on the inertial-based device.

subjects who dropped out did so within 48 h of starting the project. Anecdotal evidence provided by subjects completing the project claim it takes  $\sim 5$  days to become accustomed to ULLS. Minor transient hand tremors occurred in some subjects from each group, suggesting ambulating on crutches placed excessive demands on the hands of participants, yet the tremors were not problematic to ULLS performance. Attrition caused only 15 albuterol (8 men, 7 women), 10 placebo (6 men, 4 women), and 10 CTRL (6 men, 3 women) subjects to complete the study. Power analysis shows that, due to large effect sizes with unloading (1, 32, 33), REX (6, 46), and  $\beta_2$ -agonists (17, 28, 29), the present sample exceeds the minimal number needed for statistical computation (27). Data complied with analysis of covariance assumptions (normality, homogeneity of variance, sample independence). Only subjects who completed the study had their data analyzed. Subjects' age (mean  $\pm$  SE, REX-albuterol:  $22.5 \pm 3.1$ ; REX-placebo:  $22.6 \pm 2.0$ ; CTRL:  $25.9 \pm 6.4$  yr), body weight, and cardiovascular data (Table 1) show insignificant differences.

*Day 40* CTRL calf CSA (Table 1) and several AE strength variables (Table 2) show significant losses. CTRL *day 20* CAP, ETW, and EAP were also significantly less than *day 20* REX-albuterol values. CTRL *day 40* strength data were significantly lower than *day 40* REX-albuterol and REX-placebo values, as well as less than CTRL *day 0* results.

ETW and EAP each show group-by-time interactions. Tukey's honestly significant difference test shows that the REX-albuterol treatment evoked significantly greater *day 40* values than REX-placebo and CTRL assignments after 40 days and versus REX-albuterol *day 0* data. Thus the REX-albuterol treatment caused eccentric strength gains during a 40-day unloading period. In contrast, despite administration of the same training protocol, REX-placebo *day 40* ETW and EAP values declined significantly versus REX-placebo *day 0* results.

## DISCUSSION

Using established methods, subjects finishing the project complied with ULLS, which routinely showed the unloaded calf had a larger girth and cooler skin temperature versus the loaded calf (1, 46). The changes resulted from venous pooling and reduced muscle activity seen with unloading (1, 46). They occurred in subjects from all groups and thus were not due to albuterol. Therapeutic oral albuterol dosing has not caused adverse changes in healthy humans versus other routes of similar systemic concentration (14, 34, 35). Thus the present study's capsule dosing and ULLS were well tolerated.

Prior studies quantified AE mass and strength losses. Unloading periods of 14–17 days caused significant (9–18%) AE strength loss (1, 6, 49). A 5-wk bed rest evoked a 26% loss in AE strength (32). After 40 days, Fig. 1 suggests AE mass and strength losses are  $\sim 15$  and 25% below preunloading values, respectively (21, 32). Current CTRL AE mass and strength losses after 40 days approximate those seen after a comparable unloading duration (21, 32). Thus the current ULLS protocol, involving a lack of weight-bearing and ambulatory activity, evoked AE mass and strength losses of a magnitude comparable to prior studies.

Unloading-induced deficits receive little attenuation with concurrent aerobic training. AE strength, measured before and

Table 1. *Body mass, cardiovascular, and unloaded calf CSA data*

Variable, Units	Group	Day 0	Day 20	Day 40
Body weight, kg	REX-albuterol	72.4±3.4	72.7±3.4	72.8±3.3
	REX-placebo	74.2±3.1	73.4±3.2	72.7±3.5
	CTRL	72.7±3.4	72.5±3.2	72.2±3.5
Heart rate, beats/min	REX-albuterol	78.0±3.5	84.2±4.6	77.0±2.8
	REX-placebo	74.6±2.8	81.9±5.2	77.8±3.7
	CTRL	75.3±3.4	81.8±4.5	81.1±3.3
Systolic blood pressure, mmHg	REX-albuterol	106.0±4.6	108.0±4.1	104.0±1.8
	REX-placebo	108.8±3.4	110.2±4.0	112.2±5.4
	CTRL	108.2±4.1	109.6±4.1	108.1±4.0
Diastolic blood pressure, mmHg	REX-albuterol	68.0±7.1	69.3±6.1	73.3±2.2
	REX-placebo	76.8±4.5	72.9±6.0	76.2±3.8
	CTRL	72.4±5.4	71.6±5.1	71.2±4.8
Unloaded calf CSA, cm <sup>2</sup>	REX-albuterol	82.6±4.7	85.1±5.0	84.0±4.6
	REX-placebo	85.8±4.6	84.3±4.5	82.7±4.1
	CTRL	84.4±5.0	79.6±4.1	74.5±3.9*

Values are means ± SE. REX, resistance exercise; CTRL, control; CSA, cross-sectional area. \*Significantly ( $P < 0.05$ ) less than day 0 CTRL and day 40 REX-albuterol and REX-placebo values.

after 6 mo on Space Station MIR, still led to significant (20–48%) losses versus preflight values despite 2 h of daily in-flight treadmill and veloergometer exercise (51). In contrast, due to the more intense and site-specific mechanical loading stimulus it provides, REX offers greater strength loss attenuation (3, 4, 6, 46). During a 14-day bed rest, subjects received an every-other-day REX treatment of five sets of 6–10 calf press repetitions against isoload resistance or served as CTRL subjects (6). Results show 11% strength gains with concurrent REX, whereas CTRL subjects incurred 9% losses (6). During a 20-day bed rest, volunteers performed a 5-set 10-repetition calf press protocol on 16 days of the project or acted as CTRL subjects (3). Posttest AE CSA was unchanged due to REX, yet CTRL subjects incurred 13% losses. Isometric AE torque was also preserved with concurrent REX but declined 9% in CTRL subjects (3). During 21 days of ULLS REX done every third day, involving 2 min of isometric-isotonic AE actions, prevented mass and strength losses, whereas CTRL subjects incurred 7 and 17% deficits, respectively, to those measures (46). During a 29-day bed rest, a concurrent 4-set 14-repetition

protocol done every third day on an inertial-based REX device reduced AE atrophy 50% versus CTRL subjects (4).

Current day 0–20 AE mass and strength changes were insignificant among the groups receiving REX. Current unloaded AE mass changes were also insignificant after 20 and 40 days of ULLS due to REX. Thus training on the inertial device appears to have provided a mechanical loading stimulus comparable to most of the aforementioned short-term studies (3, 4, 46). Unlike the current and prior studies, Bamman et al. (6) noted an 11% strength gain from REX during short-term unloading. The training volume, device, and progressive resistance overload likely account for differences between Bamman et al.'s and the present and prior (3, 4, 46) results. Bamman et al. employed a greater training volume than Schulze and coworkers (46). The REX device used by Bamman et al. also permitted progressive resistance increases throughout the training period, unlike present and prior (4) studies, whereby the resistance provided by a pair of flywheels remained constant. Yet Akima and coworkers (3) used a similar REX device and set-repetition scheme as Bamman. Akima's training resistance

Table 2. *Unloaded ankle extensor strength data*

Variable, Units	Group	Day 0	Day 20	Day 40
PAV, rad/s	REX-albuterol	35.2±4.6	37.1±4.2	37.9±3.6
	REX-placebo	36.2±5.4	36.0±5.1	34.9±4.3
	CTRL	36.0±5.2	32.8±4.5	29.0±4.1‡
CTW, J	REX-albuterol	527.2±87.0	635.9±84.5	675.7±76.4
	REX-placebo	551.5±74.3	530.0±60.1	495.5±74.6
	CTRL	560.4±94.3	490.7±72.1	415.2±54.1‡
CAP, W	REX-albuterol	29.9±5.8	36.5±5.8	39.3±6.0
	REX-placebo	29.1±3.3	29.6±5.0	30.8±4.1
	CTRL	29.3±4.0	26.5±3.3§	23.6±2.7‡
ETW, J	REX-albuterol	379.3±51.9	437.9±49.5	466.7±31.1*
	REX-placebo	372.4±43.1	334.3±23.3	316.6±19.7‡
	CTRL	366.5±54.2	314.3±28.6§	289.1±15.8‡
EAP, W	REX-albuterol	20.3±3.6	24.2±3.2	25.5±3.1*
	REX-placebo	19.9±2.0	18.6±3.0	17.4±2.7‡
	CTRL	20.5±2.1	17.9±2.4§	14.9±2.3‡

Values are means ± SE. PAV, peak angular velocity; CTW, concentric total work; CAP, concentric average power; ETW, eccentric total work; EAP, eccentric average power. \*Significantly ( $P < 0.05$ ) greater than day 0 REX-albuterol and day 40 REX-placebo and CTRL values. †Significantly ( $P < 0.05$ ) less than day 0 REX-placebo value. ‡Significantly ( $P < 0.05$ ) less than day 0 CTRL and day 40 REX-albuterol and REX-placebo values. §Significantly ( $P < 0.05$ ) less than day 20 REX-albuterol value.

was determined as 70% of the maximal isometric AE torque exerted, measured four times during the 20-day study. The intent was to adjust training loads during bed rest to offer progressive overload, yet maximal isometric torque was unchanged in REX subjects, thus resistance was not increased (3). However, training loads were increased in Bamman et al.'s protocol, which induced progressive overload and AE strength gains during short-term unloading (6). In the present study, although REX preserved AE strength for 20 days in REX-placebo subjects, significant losses occurred by *day 40*. Results suggest with unloading periods longer than 20 days that the magnitude of strength losses may begin to exceed the attenuation provided by REX.

However, a recent 90-day bed rest study examined AE losses with and without an inertial-based REX protocol (5). After 90 days, CTRL subjects incurred significant mass (−29%) and strength (−37–56%) losses. REX reduced atrophy (−15%) and led to isometric-isokinetic strength losses that approximate similar changes to the current REX-placebo subjects. Yet strength testing of REX subjects on the inertial-based device showed insignificant changes versus pre-bed rest values, averaging to a mere −3.3% loss after 90 days (5). Current study REX-placebo strength changes after 40 days averaged a −7.1% decline. Differences in average percent strength loss among REX subjects in the current and 90-day studies may result from more than one factor. Per workout, the 90-day study employed nearly twice the repetitions than the present study, which likely had a greater strength loss mitigating effect. The 90-day study noted that REX preserved force despite a 15% loss in mass to suggest neural factors were altered to maintain strength (5). The role of neural factors on force output, particularly during long-term unloading, warrants further study to help delineate causes for strength loss differences despite REX administration between the present REX-placebo and 90-day studies.

Current results show that the REX-albuterol treatment significantly increased ETW and EAP after 40 days. Versus *day 0* values, the REX-albuterol treatment evoked eccentric gains despite concurrent unloading to exceed our hypothesis that the assignment would improve strength loss attenuation. Combining REX and albuterol caused greater gains than REX (3–6, 46) or albuterol (39) administered separately. Several mechanisms may explain current results. When given to animals at levels exceeding comparable human dosages, strength gains from clenbuterol result from muscle accretion via increased protein synthesis and/or decreased degradation (2, 17, 28). Yet short-term therapeutic oral albuterol dosing has not caused muscle accretion, although higher dosages (32 mg/day) added mass to a human disuse model (29). Differences in muscle mass responses between drugs may be due to the longer half-life of clenbuterol (17, 28). Due to insignificant present study calf CSA changes, REX-albuterol strength gains likely were not due to added mass. Albuterol was suggested to hasten recovery from lengthening-induced muscle soreness to improve eccentric strength (14). Yet between-group differences in postworkout muscle soreness were not observed in the present study. Other mechanisms involve greater  $\text{Ca}^{2+}$  release for greater cross-bridge formation (11, 12). Yet this may have added little to current results, since intramuscular  $\text{Ca}^{2+}$  transport is impeded by unloading (18). A potential mechanism, whereby REX-albuterol evoked ETW and EAP gains during

unloading, is through improved contractile protein sensitivity for  $\text{Ca}^{2+}$ .

Muscles show a reduced sensitivity for  $\text{Ca}^{2+}$  with unloading (7, 49).  $\beta_2$ -Agonists produce disparate effects on  $\text{Ca}^{2+}$  sensitivity that may result from the duration of treatment (36, 43).  $\beta_2$ -Agonists given to mice for 15 wk reduced  $\text{Ca}^{2+}$  sensitivity in fast-twitch soleus and extensor digitorum longus fibers (36). Yet rats given  $\beta_2$ -agonists over 15 days had the opposite effect on fast- and slow-twitch soleus fibers, with greater force output noted in weight-bearing and unloaded muscles (43).  $\beta_2$ -Agonists enhanced the affinity of fibers for  $\text{Ca}^{2+}$ , requiring less  $\text{Ca}^{2+}$  to elicit a given force output versus untreated muscle (43). This effect was greater in unloaded muscle and may have resulted from the phosphorylation of contractile proteins (43, 48). Heightened muscle fiber affinity for  $\text{Ca}^{2+}$  from albuterol administration may have compensated for reduced  $\text{Ca}^{2+}$  transport (18) and sensitivity (7, 49) to have an additive effect when combined with REX on ETW and EAP results.

Reduced muscle fatigue is another mechanism that may have augmented ETW and EAP gains. Physiological ( $10 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) clenbuterol levels in mature rats during 21 days of hindlimb suspension reduced slow-twitch fiber fatigue by 30% and lowered peak twitch and half-relaxation times to prevent the faster muscle fiber shifts seen with unloading (16). Thus current eccentric gains may have benefited from reduced fatigue provided by albuterol. Fatigue was likely a greater factor in ETW and EAP results than for PAV, a more instantaneous strength measure, to in part explain why changes to the latter variable were insignificant. Thus eccentric gains from REX-albuterol may be due to two (improved contractile protein affinity for  $\text{Ca}^{2+}$ , reduced muscle fiber fatigue) or more mechanisms that cannot be confirmed from the present study.

Unloading for 2–4 wk evoked similar magnitudes of strength loss to concentric and eccentric variables at multiple angular velocities (6, 8, 46). Bamman et al. (6) noted significant AE power losses at three eccentric (−21%) and two concentric (−14%) angular velocities; yet contractile work done at 1.05 rad/s fell 15 and 11% for shortening and lengthening actions, respectively, in CTRL subjects. After 3 wk of ULLS, isokinetic testing revealed that REX subjects incurred no concentric or eccentric force changes except when shortening at 1.05 rad/s (46). Thus unloading appears to evoke comparable strength changes to concentric and eccentric variables, both with or without REX. Yet current study concentric variables did not significantly improve from the REX-albuterol treatment. Different concentric and eccentric strength outcomes may have in part resulted from the inertial REX device. Because eccentric values are dependent on the concentric forces exerted per repetition, this likely evoked less ETW and EAP variability when strength testing on the inertial device. Table 2 data appear to support this idea, because ETW and EAP variability is less versus corresponding concentric measures. Prior studies also noted that REX-albuterol treatment led to greater eccentric gains (13, 14). Isokinetic dynamometry was used for both strength testing and training, and, like the inertial device, concentric forces dictate the magnitude of eccentric resistance (13, 14). Thus, perhaps due to reduced data variability, eccentric variables note a greater likelihood of significant changes on such strength-testing devices. Continued research with different testing modes may help determine

whether REX-albuterol has a greater impact on eccentric strength.

Side effects with clenbuterol are preceded by significant blood pressure and heart rate shifts (17, 47). Yet Table 1 data show insignificant changes, suggesting that albuterol appears to have been well tolerated by the current subjects. Side effects with albuterol vary by dosage and route of administration. Hand tremors are a benign and common side effect due to peripheral  $\beta$ -agonist receptor binding within the forearm and not from changes to the heart or other organs (31). More adverse effects, such as heart palpitations and hypokalemia, occur when high albuterol doses are given intravenously (30, 42). Palpitations and heart rate increases are due to greater cardiac ( $\beta_1$ ) and peripheral blood vessel ( $\beta_2$ ) receptor activity resulting from intravenous albuterol therapy that in turn causes vasodilation-induced drops in diastolic blood pressure (30). To compensate, heart rates increase from reduced parasympathetic drive (30). Hypokalemia from intravenous albuterol dosing is transient and follows greater  $\text{Na}^+$ - $\text{K}^+$  ATPase activity, yet supplemental  $\text{K}^+$  is not required (42). Oral (2–8 mg) albuterol in adults caused insignificant heart rate and blood pressure changes (24, 31). A 1 mg/kg oral albuterol dose in guinea pigs did not elevate heart rate, yet at 5 mg/kg heart rates increased 30 beats/min (20). Yet such a dose, expressed relative to body mass, far exceeds human dosages. At 16 mg/day, current subjects ingested on average a mere 0.22 mg/kg of albuterol daily.

A lack of adverse effects with oral albuterol may result from its rapid sulfation and low systemic availability, as low drug plasma levels occur despite quick serum absorption (34). Another possible reason for the lack of current study adverse effects is with chronic controlled-release oral albuterol (4 mg, 4 times daily), steady-state plasma levels occur by day 3 of dosing (42). Table 1 shows that the REX-albuterol treatment did not cause significant heart rate and blood pressure changes to address a current study hypothesis. ETW and EAP gains suggest AE strength may be maintained for unloading periods beyond 40 days due to the REX-albuterol assignment. Continued research identifying the mechanisms responsible for the strength gains incurred during unloading, as well as the safety of REX-albuterol administration in other models, is warranted.

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